Spatial variability of oceanic phycoerythrin spectral types derived from airborne laser-induced fluorescence emissions

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We report spatial variability of oceanic phycoerythrin spectral types detected by means of a blue spectral shift in airborne laser-induced fluorescence emission. The blue shift of the phycoerythrobilin fluorescence is known from laboratory studies to be induced by phycourobilin chromophore substitution at phycoerythrobilin chromophore sites in some strains of phycoerythrin-containing marine cyanobacteria. The airborne 532-nm laser-induced phycoerythrin fluorescence of the upper oceanic volume showed distinct segregation of cyanobacterial chromophore types in a flight transect from coastal water to the Sargasso Sea in the western North Atlantic. High phycourobilin levels were restricted to the oceanic (oligotrophic) end of the flight transect, in agreement with historical ship findings. These remotely observed phycoerythrin spectral fluorescence shifts have the potential to permit rapid, wide-area studies of the spatial variability of spectrally distinct cyanobacteria, especially across interfacial regions of coastal and oceanic water masses. Airborne laser-induced phytoplankton spectral fluorescence observations also further the development of satellite algorithms for passive detection of phytoplankton pigments. Optical modifications to the NASA Airborne Oceanographic Lidar are briefly described that permitted observation of the fluorescence spectral shifts.

OCIS codes: 010.3640, 010.4450, 300.2530, 300.6450, 300.6280.

1. Introduction

Since 1979, laser-induced phycoerythrin (PE) fluorescence has been observed from airborne platforms, specifically the NASA Airborne Oceanographic Lidar (AOL).[1] Until now, phytoplankton fluorescence in the yellow band (580-nm region) was ascribed only to the general PE pigment class,[2] and it has been used as a diagnostic signal for the presence of marine cyanobacteria, cryptophytes, or both in surface waters. PE exists in a number of distinct spectral forms, however, depending on species and photoacclimative state. These spectral types tend to be segregated in the oceans across inshore–offshore gradients related to nutrient status and clarity of the water.[3,4] Remote measurement of the spatial variability of these spectral types by use of the AOL was a goal of the present research. (A long-term goal of this research is to use such spectral fluorescence emissions in the development of satellite retrieval algorithms, eventually leading to global oceanic speciation mapping in the surface layer.)

PE is a class of pigment-protein macromolecule with chromophores that absorb light in the 480–560-spectral region. In cyanobacteria the functional light-harvesting unit is a hexameric protein aggregate (sometimes associated with an additional pigment-protein monomer) that contains 34 to 38 covalently bound chromophores. These chromophores are of two types: phycoerythrobilin (PEB), which absorbs in the 550–565-nm region, and phycourobilin (PUB), which absorbs in the 495-nm region. PEB is necessary for energy transfer between units of the photosynthetic light-harvesting antenna and is therefore found in all PE-containing marine cyanobacteria. Some strains of cyanobacteria have PUB substituted at selected chromophore binding sites on the PE molecule, conferring greater light absorption in the blue-green region of the spectrum. A high degree of PUB substitution with dominant absorption peaks at 495 nm is notable in specific

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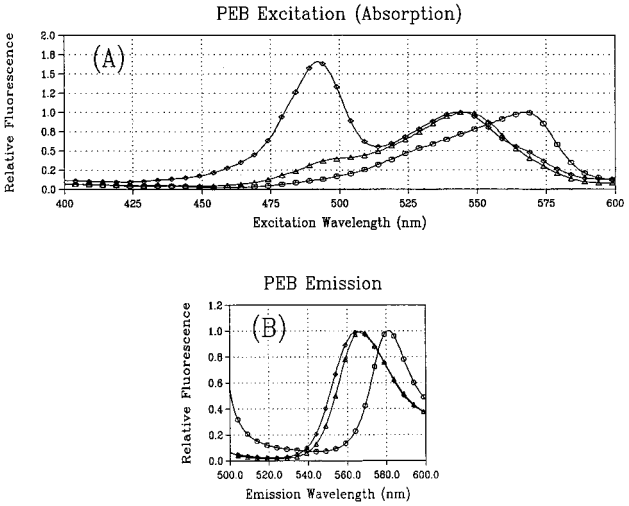

Fig. 1. PEB excitation spectra and fluorescence emission spectra of three cultures of phytoplankton that have PE: PUB rich (WH 8102; diamonds), PUB intermediate (WH 7803; triangles), and PUB deficient (WH 8018; circles). (A) The peak of the excitation spectrum shifts toward blue wavelengths when PUB chromophores are substituted within the hexameric protein aggregate. (B) As with the excitation spectra, the peak of the fluorescence emission spectrum shifts toward blue wavelengths when PUB chromophores are substituted within the hexameric protein aggregate.

strains of the ecologically important marine cyanobacterium genus *Synechococcus*.[5] The PE spectral absorption band peaks near 565 nm when only PEB chromophores are present [Fig. 1(A)]. The presence of PUB chromophores (with absorption-band peak at 495 nm) causes a 10–15-nm blueshift in the peak wavelength of the PEB absorption spectrum. Specifically, for PUB-containing PE the PEB absorption band occurs near 550 nm, regardless of the relative amounts of PUB and PEB [Fig. 1(A)]. Notice that 532-nm laser emission can be absorbed by both PEB and PUB chromophores (though not optimally by either).

PE fluorescence, regardless of the presence or absence of PUB, is always redshifted relative to the PEB absorption peak, indicating that only PEB chromophores fluoresce[5] [Fig. 1(B)]. For PUB-deficient PE, the fluorescence emission band of PEB peaks at ~580 nm [Fig. 1(B)]. However, the peak fluorescence emission wavelength from PUB-containing PE is blueshifted [Fig. 1(B)]. This spectral shift in fluorescence emission provides the possibility of identifying spectral strains by means of a single-wavelength excitation lidar system by measurement of the emission spectra of PE fluorescence.

Recent modifications to the original receiver optics of the AOL have resulted in significant rejection of backscattered 532-nm radiation accompanied by a substantial reduction of scattered light within the spectrometer. These improvements now permit observation of laser-induced fluorescence in the 560–570-nm band, thus facilitating observation of the shorter-wavelength emission of PUB-rich cyanobacteria. We report observations of airborne laser-induced fluorescence associated with PE containing different levels of PUB. The PUB and PEB spectral variations were observed during flights conducted over coastal, shelf, slope, Gulf Stream, and Sargasso Sea water masses of the western North Atlantic Ocean in early April 1995.

2. Materials and Methods

A. Airborne Data Acquisition

The requisite data were acquired on 3 April 1995 with the AOL flown aboard the NASA Goddard Space Flight Center P-3B aircraft. The laser-induced fluorescence measurement methods were analogous to those already published.[6,7] Briefly, a 532-nm laser pulse is transmitted vertically downward into the ocean to induce phycoerythrin and chlorophyll fluorescence emission from waterborne phytoplankton and water Raman emission from the surrounding seawater molecules.

The concurrent phycoerythrin (~540–595-nm), chlorophyll (~670–690-nm), and water Raman (~645-nm) spectral emissions are collected by a telespectroradiometer. The original AOL system[8–12] was modified to provide rejection of the 532-nm laser excitation wavelength from the instrument spectrometer and substantial reduction of scattered light. Specifically, a narrow-band holographic notch transmission filter placed into the collimated segment of the light path effectively rejects backscattered 532-nm laser radiation from the spectrometer. (The 532-nm pulse reflected from the holographic notch filter is in turn viewed by a photomultiplier and is used to temporally define the ocean surface target and initiate digitization of the fluorescence spectra.) Additional modifications include the removal of rigid light guides and all turning mirrors that constitute the original optical axis. A fiber optic face plate now occupies the focal plane of the new in-line optical path and transports the spectral radiation to mechanically reconfigured banks of original photomultiplier tubes. The resolution of each of the 84 channels of the fiber optic faceplate system is ~4 nm. However, the number of photomultiplier tubes currently available within the redesigned spectrometer is only 28; therefore we optically combined groups of three fiber optical channels at each photomultiplier tube to achieve ~12-nm resolution per output band for this experiment. The signal path from the photomultiplier tubes through and including digitization remain essentially the same as reported previously.[8–12] Compared with the original light guides, the fiber optic channels have superior scattered-light rejection attributed to a considerably smaller viewing or acceptance angle.

A spectral and radiometric calibration is performed before and after each flight mission by viewing an internally illuminated 0.75-m-diameter calibration sphere[13] placed beneath the aircraft telescope viewing port. Immediately following the 0.75-m sphere calibration, a 10-cm-diameter calibration sphere within the AOL system is viewed by mechanical in-

troduction (at the focal plane of the telescope) of the radiation via fiber optics. The small calibration sphere permits immediate transfer of the 0.75-m sphere calibration into the aircraft domain for periodic in-flight calibration. The 10-cm sphere calibration is followed by viewing 40-ns pulsed radiation from a bank of red LED's located behind the diffraction grating and in front of the fiber optic faceplate. The pulsed LED's provide transfer of the ground (and onboard) dc tungsten lamp calibrations to the wide-bandwidth pulsed portion of the AOL detection–amplification–digitization system.

The redesigned AOL fluorosensor is flown along with several additional sensor components and subsystems. These include two infrared Heimann KT-19 radiation thermometers to measure sea surface temperature (SST), a down-looking 256-channel spectroradiometer to gather ocean color radiance, and an uplooking 256 channel spectroradiometer equipped with a cosine collector to acquire downwelling solar irradiance. Both of the auxiliary spectroradiometers are set to provide spectral coverage between ~400 and ~720 nm. The SST profile data acquired with the Heimann radiometers are presented in Section 3 below to aid in the interpretation of the phycoerythrin fluorescence profiles. The ocean color radiance and solar irradiance data gathered with the two auxiliary spectroradiometers are not included in this paper but will be used in follow-on studies with the laser-induced phycoerythrin fluorescence data.

B. Laboratory Procedures

In vivo fluorescence excitation and emission spectra were determined on cultivated *Synechococcus* clones containing PE with high, medium, or no PUB chromophores typical of three spectral classes of marine *Synechococcus*. Clones were obtained from the Woods Hole culture collection and grown at ~50 μE m^{-2} s^{-1} and 20 °C in F/2 (-Cu) media with a Sargasso Sea water base. Fluorescence measurements were made on an SLM/Aminco Model 500C spectrofluorometer with 4-nm excitation–emission bandpasses, corrected emission signals, and excitation ratio mode.

3. Results

A. Laboratory Observations

Fluorescence excitation and emission spectra for three *Synechococcus* strains are shown in Fig. 1. These strains represent maximum and minimum PUB/PEB ratios (and a typical intermediate form found among numerous isolates of *Synechococcus* from around the world). Laser light (532 nm) excites all spectral forms, mainly by exciting the blue absorption tail of PEB. PUB absorption is weak at 532 nm.[5] Because of the possibility of mixtures of spectral types in the water column and variations in quantum yield of PE fluorescence owing to photoacclimative and nutrient response processes,[14,15] quantitative estimates of PE based on spectral fluorescence are imprecise. However, it is possible to develop an index of the relative occurrence of PUB-containing forms by quantifying the ratio of fluorescence in the short- and long-wavelength emission bands.

The sensitivity of this index to PE with and without PUB can be seen in the separation between the two fluorescence emission bands (Fig. 1). With the new capability of the AOL to resolve the 566-nm band, it is possible to detect the emission band of PUB-containing organisms, which yield a significantly higher ratio of the 566–593 optical channels than that obtained from PE lacking PUB. Thus the spectral fluorescence emission of PE-rich *Synechococcus* cultures suggests that naturally occurring species that have PUB can potentially be distinguished from PUB-lacking species by use of airborne laser spectrometry. The actual performance of a 532-nm source for defining relative PEB and PUB amounts is not easily determined from an analysis of laboratory excitation and emission spectra of cultures that contain PEB and PUB. Airborne field results are a better indicator of the potential effectiveness of the laser source and the entire methodology.

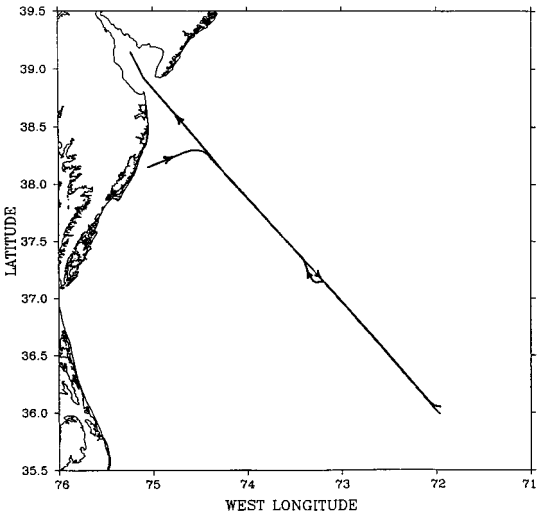

Fig. 2. Flight tracks of the NASA P3-B aircraft with the AOL system on 3 April 1995. The flight was initiated from Wallops Island toward the northeast, reoriented ~90°, and directed southeast to ~72 °W longitude and ~36 °N latitude. The flight track then traversed toward the northwest and terminated within Delaware Bay. The flight track deviation at 73.25 °W longitude and ~37.25 °N latitude was made to avoid a military field operation that was being conducted in that vicinity.

B. Airborne Observations

Figure 2 shows the flight track of the AOL on 3 April 1995. The flight was initiated from Wallops Island toward the northeast, reoriented ~90°, and directed southeast to 72 °W longitude and 36 °N latitude. There the flight track was reversed and traversed toward the northwest and terminated within Delaware Bay. The flight track deviation near 73.25 °W longitude and 37.25 °N latitude was made to avoid a

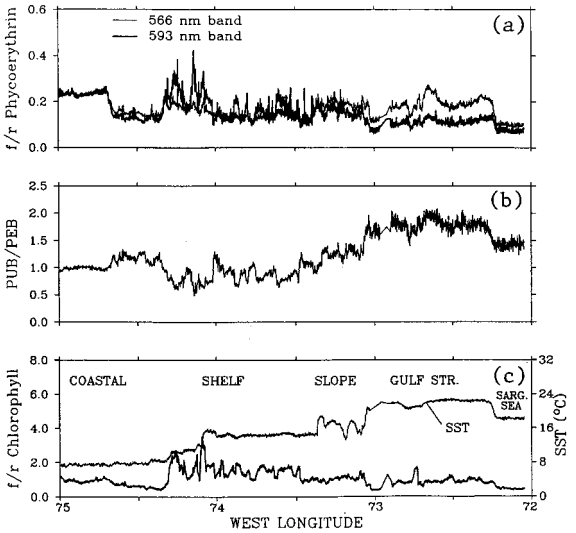

Fig. 3. (a) 532-nm laser-induced and water Raman–normalized PE fluorescence in the 566- and 593-nm AOL bands acquired during the west-to-east (outbound) flight track. (b) Ratio of the 566-nm fluorescence to 593-nm fluorescence. In the slope waters at ~73.4 °W longitude the 566/593 nm ratio begins increasing in the offshore direction. (c) 532-nm laser-induced and water Raman–normalized chlorophyll fluorescence acquired during the outbound flight track. The chlorophyll fluorescence is highly correlated with the PEB fluorescence within the shelf water mass, except within the colder water flanking the coastline at the western end of the flight track. The SST data are from the airborne infrared radiometer.

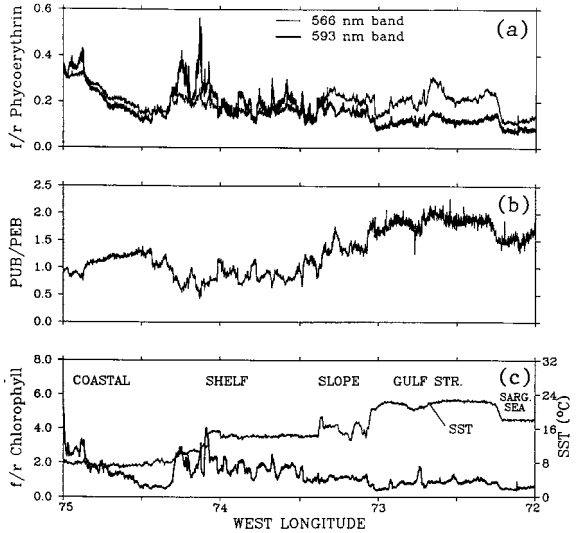

Fig. 4. (a) 532-nm laser-induced and water Raman–normalized PE fluorescence in the 566- and 593-nm bands acquired during the east-to-west (inbound) flight track. (b) Ratio of 566-nm fluorescence to 593-nm fluorescence. (c) 532-nm laser-induced and water Raman–normalized chlorophyll fluorescence acquired during the inbound flight track. The chlorophyll fluorescence is highly correlated with the PEB fluorescence within the shelf water mass. The SST data are from the airborne infrared radiometer.

military field operation that was being conducted in that vicinity.

Figure 3(a) shows the 532-nm laser-induced PE fluorescence in the 566- and the 593-nm bands (both bands have a width of ~12 nm) acquired during the west-to-east (outbound) flight track. (The 593-nm band was used in lieu of the 580-nm band to provide wider spectral separation.) The ratio of the fluorescence signal in the 566-nm band to that in the 593-nm band is plotted in Fig. 3(b), and profiles of the SST and laser-induced chlorophyll fluorescence are shown in Fig. 3(c) to aid in the interpretation of the PE fluorescence profiles. Figure 4 contains the corresponding profiles acquired during the east-to-west (inbound) flight track. (The laser-induced PE and chlorophyll fluorescence bands have been normalized by the water Raman signal at ~645 nm to remove variability in attenuation properties within the upper portion of the water column.[1,16]) One can readily see the precision or high measurement reproducibility of the AOL fluorosensor by visually comparing the fluorescence profiles from the outbound and the inbound flight tracks in Figs. 3 and 4, respectively. The locations of even relatively fine features are apparent in the contrasted profiles captured with the aircraft flying in the opposite direction. [The western portions of profiles should not be directly compared with one another west of 74.3 °W because the flight paths were not overlapping (see Fig. 2).] The locations of the coastal, shelf, slope, Gulf Stream, and Sargasso Sea water masses have been labeled in both Figs. 3 and 4.

The along-track profiles of the PE fluorescence bands [Figs. 3(a) and 4(a)] suggest the presence of both PUB and PEB pigments over the entire flight line. However, the relative strength of the laser-induced PE fluorescence in the 566- and 593-nm bands varies systematically. This systematic variability is especially apparent in Figs. 3(b) and 4(b), in which the 566-nm fluorescence has been ratioed to the 590-nm fluorescence. The intensity of fluorescence attributed to PUB-containing PE is seen to be elevated over the slope, Gulf Stream, and Sargasso Sea regions, whereas the intensity of fluorescence from PUB-lacking PE appears highest over the shelf region.

These airborne and laboratory observations are consistent with known distribution patterns for the two pigments; i.e., phytoplankton containing higher portions of the PEB pigment are generally associated with coastal waters, whereas the PUB-bearing organisms, in general, are the dominate PE pigment in offshore, oligotrophic waters.[3,4] The chlorophyll fluorescence can be seen to covary with the PEB pigment over most of the flight line, except for the easternmost portion of the outbound survey. In the inbound survey (Fig. 4) the strong correspondence between chlorophyll and PEB fluorescence continues within the eastern portion of the flight line at the entrance to Delaware Bay.

4. Discussion

Laser-induced fluorescence from the phycoerythrin PEB pigment fluorescence has been remotely ob-

served since 1979.[1] However, the presence of 532-nm laser light scattered into the fluorosensor spectrometer resulted in masking the presence of shorter-wavelength PE fluorescence. Recent modifications to the fluorosensor spectrometer (especially the implementation of a narrow-band holographic notch filter, use of fiber optics instead of light guides, and elimination of turning mirrors) have resulted in almost complete rejection of backscattered laser light and a substantial decrease in all scattered light. Laser-induced PE fluorescence spectra acquired with the AOL over coastal, shelf, slope, Gulf Stream, and Sargasso Sea waters of the Middle Atlantic Bight during early April 1995 contain an ~12-nm band centered at 566 nm, which is distinct from the band centered at 593 nm. The capability to measure remotely the spectral shift associated with PUB-containing PE suggests a potential application of airborne laser fluorescence methodology to wide-area mapping of the relative spatial variability of PE. This application may find more use in coastal–midshelf regions (and in coastal–oceanic transition zones) where the relative presence of the chromophoric types would be expected to be more variable, as can be seen from the April 1995 airborne fluorescence data set.

The excitation spectra of cultures (Fig. 1) and the excitation spectra of filtered surface samples[17] strongly suggest that the best bands for passive (solar) detection of PUB and PEB absorption are ~495 nm for PUB and ~555 nm for PEB. However, an important application of airborne wide-area fluorescence variability is the actual development of algorithms to retrieve the PUB and PEB absorption coefficients from water-leaving radiance data. Application of such algorithms to satellite ocean color imagery could lead to global PUB and PEB pigment maps similar to the chlorophyll scenes produced from Coastal Zone Color Scanner data. For example, concurrently observed water-leaving radiances can be used to identify optimum bands for retrieving PUB and PEB pigment absorption by application of methods similar to active–passive correlation spectroscopy.[18] Also, optimum PUB and PEB absorption band locations can potentially be improved by use of the airborne laser PE fluorescence data (together with concurrent chlorophyll and chromophoric dissolved organic matter laser-induced fluorescence data[6,7] within radiance models[19]). Once the optimum color band locations have been identified, the PUB and PEB absorption coefficients can, at least in principle, be retrieved from radiance or reflectance models.[20] Finally, the arduous task of establishing valid specific absorption coefficients for PUB and PEB must be accomplished before the absorption coefficients of these pigments can be converted into concentrations.

The PUB and PEB identification algorithm herein uses only two bands, located on the bluest and reddest portions of the PE fluorescence emission spectrum. Future efforts will investigate the use of other algorithms, including the centroid algorithm. That algorithm calculates the centroid of the entire PE emission band. The centroid shifts to bluer wavelengths in the presence of additional PUB. Comparative analyses will be used to determine whether PUB and PEB identification is improved.

The passive observation of PE pigment by active–passive (laser–solar) correlation spectroscopy methods[18] has been reported.[2] Now, following laser observations of the PEB variability, the potential exists to investigate the feasibility of detecting and eventually quantifying both PUB and PEB pigment absorption coefficients by use of oceanic water-leaving radiances. The presence of both PUB and PEB absorption within water-leaving radiances will be established as before, except that both the PUB and the PEB pigments will now be sought by correlation spectroscopy[18] methods. These analyses are beyond the scope of this paper, and future investigations will address the potential detection of both PUB and PEB pigments by use of oceanic upwelled radiances.

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